

Contents

1. Description
 - 1.1 Background information
 - 1.2 Reagent and instrument requirements
2. Protocol for homogenization of tissue for total RNA isolation

1. Description

1.1 Background information

The isolation of subcellular material such as total RNA from tissues or cells requires fast and thorough homogenization of the respective starting material. The gentleMACS™ Dissociators provide optimized programs that meet these requirements. In combination with M Tubes, the gentleMACS Dissociators allow the automated homogenization of tissues in a closed system, enabling sterile sample handling. A single sample or two samples can be processed in parallel.

1.2 Reagent and instrument requirements

- gentleMACS Dissociator (# 130-093-235)
- gentleMACS Octo Dissociator (# 130-095-937)
- gentleMACS M Tubes (# 130-093-236, # 130-096-335)
- Total RNA isolation kits from different suppliers

2. Protocol for homogenization of tissue for total RNA isolation

▲ The protocol has been tested successfully for a broad range of tissues such as liver, lung, brain, spleen, kidney, muscle, hypothalamus, intestine, bladder, heart, or skin.

▲ **Note:** Very hard material such as bone should not be processed since it may damage the M Tubes.

▲ The sample volume should be between min. 350 µL and max. 10 mL of lysis buffer.

▲ Its molecular characteristics make RNA chemically unstable and inherently susceptible to ubiquitous RNases. It is therefore recommended to rapidly lyse samples in Lysis/Binding buffer without interruptions to minimize RNA degradation. Avoid thawing of frozen samples before lysis.

▲ For details on the use of the gentleMACS Dissociators, refer to the gentleMACS Dissociator user manuals.

1. Choose one of the following gentleMACS Programs:
For fresh tissue: gentleMACS Program **RNA_01**
For frozen tissue: gentleMACS Program **RNA_02**
2. Adjust lysis buffer to room temperature.
3. According to the kit manufacturer's recommendations pipette an appropriate amount of lysis buffer provided by the total RNA isolation kit into the M Tube.
4. Transfer tissue sample into the Lysis Buffer in the M Tube.
▲ **Note:** Place sample directly into the buffer to avoid adherence of the tissue to the tube wall.
5. Tightly close M Tube and turn the tube upside down in one quick move ensuring that the sample material reaches the area of the rotor/stator.
6. Attach it upside down onto the sleeve of the gentleMACS Dissociator.
7. Run one of the following gentleMACS Programs:
For fresh tissue: gentleMACS Program **RNA_01**
For frozen tissue: gentleMACS Program **RNA_02**
8. After termination of the program, detach M Tube from the gentleMACS Dissociator.
9. (Optional) For sample volumes below 3 mL or if excessive foam formation occurred during the homogenization process, centrifuge M Tube at 2000×g for 1 minute to collect lysate at the tube bottom.
10. Remove the homogenized sample from the tube.
▲ **Note:** Homogenized tissue can be removed from the closed M Tube by pipetting through the septum-sealed opening in the center of the cap of the M Tube. Use ART 1000 REACH 1000 µL pipette tips.
11. Proceed with total RNA isolation according to the kit manufacturer's recommendations.

All gentleMACS Protocols are available at www.miltenyibiotec.com.

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